FISHERY RESEARCH

MONTHLY VARIATION IN PREVALENCE AND INTENSITY OF *MYXOBOLUS CEREBRALIS* INFECTIONS IN SENTINEL RAINBOW TROUT EXPOSED TO RIVER WATER SUPPLIES OF SAWTOOTH AND PAHSIMEROI HATCHERIES

February 1, 2000—January 31, 2001

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ABSTRACT

Myxobolus cerebralis has been demonstrated in the upper Salmon River and Pahsimeroi River since 1987 and can be detected in fish cultured at Sawtooth and Pahsimeroi hatcheries. These facilities are operated by the Idaho Department of Fish and Game as mitigation for hydroelectric projects on the Snake River. Both hatcheries use river water to culture chinook salmon *Oncorhynchus tshawytscha* for a portion of the 18 months required to attain smolt stage. The seasonal effect on the prevalence and intensity of infection of *M. cerebralis* has not been documented at these facilities.

Exposure trials for *M. cerebralis* were conducted monthly from February 2000 through January 2001 by placing live-boxes containing 20 sentinel rainbow trout *O. mykiss* in the river water supplies of the Sawtooth and Pahsimeroi hatcheries. An additional exposure site was placed at the effluent of the Pahsimeroi Hatchery settling pond. The river discharge and temperatures were monitored during exposures trials. After 10 d, sentinel trout were transported back to the Eagle Fish Health Laboratory, held until 1,800 Celsius temperature units had accumulated post exposure, sacrificed, and analyzed for prevalence and intensity of infection of the parasite.

Intensity of infection and prevalence were compared to site location. Exposure to the parasite appeared to be greatest in the months of April and May, with a second peak in August at the Sawtooth Fish Hatchery. Pahsimeroi Hatchery displayed a similar pattern with peaks in April and August. The settling pond site at the Pahsimeroi Fish Hatchery had a single peak of infection during June and July. An infection of *M. cerebralis* was not established from Salmon River water at Sawtooth Fish Hatchery during the months of February and March of 2000, but *M. cerebralis* was detected in every month of the trial at both Pahsimeroi Fish Hatchery exposure sites.

We recommend expanding the quantity of specific pathogen-free well water available at both the Sawtooth Fish Hatchery and Pahsimeroi Hatchery to facilitate culture strategies to avoid *M. cerebralis* infection. Further recommendations for these facilities were suggested to limit MC activity. The data generated by this study has been used to limit exposure of juvenile chinook salmon to *M. cerebralis* and to justify the investment in expanded pathogen-free well water supplies for both facilities.

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INTRODUCTION

Myxobolus cerebralis (MC), the etiologic agent of salmonid whirling disease (WD), is a myxosporean parasite that infects most salmonids and has been implicated as a factor in the decline of wild trout populations in Montana (Nehring and Walker 1996) and Colorado (Vincent 1998). The parasite selectively infects cartilage and causes skeletal deformity, scoliosis, black-tail, and whirling behavior. The effects of infection range from sub-clinical to acute disease with mortalities in fry and fingerlings (Hnath 1983).

The complex life cycle of MC has been thoroughly described in the literature (Hedrick and El-Matbouli 2002). Susceptibility to the infective triactinomyxon (TAM) life stage is dependent on species, age, size, site, and challenge dose. The greatest susceptibility to clinical disease is in young fish whose skeletons are mostly comprised of cartilage. As the skeleton ossifies to bone, clinical damage by the parasite is less dramatic. Temperatures and seasonal changes can affect transmission and severity of disease (Vincent 1998).

To date, the parasite has been detected in 21 river drainages throughout Idaho (Cavender et al. 2003); these include the water sources for three Idaho Department of Fish and Game (IDFG) fish hatcheries: Hayspur Fish Hatchery, Pahsimeroi Fish Hatchery (PH), and Sawtooth Fish Hatchery (SFH). Hayspur Fish Hatchery is funded by IDFG license dollars and is IDFG's major source of rainbow trout *Oncorhynchus mykiss* eggs. The presence of MC was confirmed in 1988, and the hatchery has since undergone substantial modifications, investing approximately $1.2 million, to exclude this parasite from its water supply. A physical renovation in 1995 established new wells for a specific pathogen-free (SPF) water source, and a change of mission eliminated the hatchery's catchable trout program. Prior to renovation, the catchable trout reared at this facility were exposed to MC by using Loving Creek (a confirmed positive MC source) as a water supply. By eliminating this program, MC positive trout are no longer stocked into Idaho waters for angling.

The presence of MC was first detected in summer chinook salmon *O. tshawytscha* at PH in 1987, and a strategy to avoid MC infection, by rearing PH chinook salmon at SFH on well water, has been implemented since 1994. A cooperative effort between IDFG and Idaho Power Company to renovate the hatchery and provide a well water supply for early rearing has stalled because of limited budget and water rights issues. Completion of this renovation project would provide PH with enough well water to raise chinook salmon to a size greater than 75 mm fork length and reduce the impact of MC infection. As a result, PH would become independent of SFH for chinook salmon production.

As with PH, SFH also experienced its first MC detection in 1987 in spring chinook salmon and began avoidance strategies to lessen the impacts of MC. The SFH is operated under the Lower Snake River Compensation Plan (LSRCP). The LSRCP administration has raised the priority of expanding SPF well water supplies at SFH, but the renovation work is also limited by budget constraints. The SFH and PH were chosen as study locations for this investigation because these facilities have not yet received major physical renovations.

Both SFH and PH managers developed rearing strategies for chinook salmon to avoid exposure to the parasite during life stages most susceptible to infection. Efforts have primarily centered on rearing fish in well water until they reach 70 to 90 mm in length. This does not prevent eventual infection but greatly reduces clinical infection of the host. The advantage of this rearing strategy was that it was an effective change that did not require substantial capital investment. Prior to 1994, chinook salmon at SFH were initially reared on SPF well water and then transferred to the Salmon River water supply as early as March. The fish were then kept in concrete raceways until release for migration the following March or April. After 1994, Sawtooth chinook salmon were held on well water as late as November before being transferred to the outside concrete raceways supplied with water from the Salmon River. These fish were reared in this environment until release the following April. The results of preliberation sampling since implementation show a modest reduction in prevalence from 10.8% to annually 2.9% (IDFG database 2003).

Prior to 1994, chinook salmon were introduced as fry to the small raceways at PH for early rearing from November to February. The transfer of these chinook salmon for final rearing in the earthen ponds was completed in April or early May. The fish were exposed to Pahsimeroi River water at both locations. Beginning in 1994, protocols were developed to delay exposure of chinook salmon to river water until fish reached a minimum size of at least 75 mm fork length. This was accomplished by transferring eggs from PH to SFH for incubation and initial rearing on well water. These fish were then transferred to Salmon River water at SFH by June and subsequently transferred back to PH from September to November for final rearing in the earthen ponds at the upper facility until release. Results from preliberation sampling conducted every spring since 1995 indicate this practice has reduced the prevalence of MC in PH chinook salmon smolts from 48.2% to 10.6% (IDFG database 2003). Thus, it appears the Pahsimeroi River water supply became positive for TAMs earlier and at a higher intensity level than the Salmon River water supply at SFH.

Whirling disease detection prevalence (collected from returning chinook salmon adults 1987 to 2003) during routine fish health sampling at spawning demonstrates a declining trend at both SFH and PH (IDFG database 2003). A noticeable decline occurred from 1996 to 2003, when fish returning to these hatcheries were cultured to smolt stage utilizing strategies to avoid MC infection. The prevalence of MC in returning anadromous adults has remained below 10% since 1996. As noted above, data collected from SFH and PH juveniles at preliberation sampling demonstrates a decline in MC prevalence once avoidance strategies were implemented.

Other reasons we suspect that there may be differences in prevalence and intensity at SFH and PH is that there are notable differences in the environments of the two valleys upstream of these facilities. Neither river system is influenced by reservoirs, but the Pahsimeroi River has a peak hydrograph in December, while the Salmon River peaks in June. The Pahsimeroi River experiences a reverse hydrograph (e.g., low flows in spring and higher flows in fall and winter) due to the arid conditions of this part of the state, extensive ground water springs, and the withdrawal of water for irrigation. It is common for portions of the Pahsimeroi River to be dewatered from irrigation activities during spring and summer months. The Pahsimeroi River valley economics are based on traditional ranching methods that have been implicated in causing extensive siltation and riparian zone degradation. In contrast, the upper Salmon River area has limited livestock grazing, low silt production, and the riparian zone is in good condition. Since the Pahsimeroi River has more silt and livestock impact, it is more suitable habitat for tubificid worms. The Pahsimeroi River is strongly influenced by springs, while the upper Salmon River has little spring activity. These springs in the Pahsimeroi River may provide a constant temperature environment that produces TAMs throughout the year.

The production goals at SFH are set by mitigation agreements that impact the time of entry into river water and are driven by volume limitations of well water and the number of chinook salmon being reared. The quantity of chinook salmon eggs taken each year determines (and drives the decision process) how long each group of salmon remains on well water at SFH. Currently, SFH spring chinook salmon and Redfish Lake sockeye salmon *Oncorhynchus nerka* are given priority over the Pahsimeroi chinook salmon in water management at the SFH (e.g., priority to incubate and rear on well water). In years of excess adult chinook salmon returns, the Shoshone-Bannock Tribe's egg box program at SFH has a higher priority than the Pahsimeroi chinook salmon program at SFH. Because of this decision process, Pahsimeroi Hatchery chinook salmon are the first fish at SFH to be reared on Salmon River water, thus increasing exposure to MC. Standards of culture parameters, determined by the Integrated Hatcheries Operations Team (IHOT), are expressed in the Best Management Practices (BMP) and also impact the timing that chinook salmon are exposed to MC.

The effects of MC infection on the chinook smolt-to-adult return rates and contributions to delayed mortality are unknown. Perturbations in the data make it difficult to sort out MC's role in producing delayed mortality in chinook salmon during migration. While MC is most likely not a large contributor in delayed mortality of chinook salmon, its exact impact is undetermined. The information gathered in these exposure trials will give insight on the impacts of MC exposure timing and duration on chinook salmon juveniles at SFH and PH. This will enable the hatchery managers to pick the best possible time to start rearing fish on river water so they can reduce the prevalence and intensity of MC infection in the fish they release. Experiences in other states have demonstrated that not planting MC positive rainbow trout or closing facilities with positive populations can reduce the level of MC in wild populations to below the limits of detection (Modin 1998). Closing PH and SFH or reducing the numbers of anadromous fish these facilities release are not options available to IDFG. Reduction in infection levels in the fish that are released must benefit both the hatchery production and the aquatic ecosystems involved.

STUDY SITE

This study was conducted at SFH and PH; both facilities are located in Central Idaho (Appendix A). Sawtooth Fish Hatchery was built in 1985 by the Army Corps of Engineers as part of the LSRCP. This facility is funded by the Bonneville Power Administration through the U.S. Fish and Wildlife Service and operated by the IDFG. The SFH is located eight kilometers (five miles) south of Stanley, Idaho (UTM 11, 669592E, 4890183N). The adult weir that transects the Salmon River is approximately 695 km (417 river miles) from Lower Granite Dam and 1442 km (865 river miles) from the mouth of the Columbia River. The LSRCP objective of this facility is to return 19,000 adult chinook salmon and 25,000 adult steelhead *Oncorhynchus mykiss* to the project area upstream of Lower Granite Dam. The SFH utilizes river water from the Salmon River to service 6 small raceways and 14 large raceways that are used to culture chinook salmon from fry to release. The river water temperatures range from 0°C in the winter to 20°C in the summer. In addition, three wells at this facility can provide approximately 8 cfs of well water to service 14 concrete vats and 16 semicircular tanks. The well water temperatures range from 4°C in the winter to 11°C in the summer. The live-box exposure site for this facility was in the head box of small raceway four (Appendix B) provided with Salmon River water.

The PH was built in 1967, is owned and funded by Idaho Power Company, and is operated by IDFG. The hatchery is located near Ellis, Idaho, 1.6 km (one mile) upstream of the confluence of the Salmon River and Pahsimeroi River (UTM 11,734637E, 4952129N). The Pahsimeroi River enters into the Salmon River approximately 132 km (79 river miles) downstream of the SFH. The earthen rearing ponds are located approximately 11.5 km (7 river miles) upstream of the main hatchery on the Pahsimeroi River. The objectives of this facility are to raise 1,000,000 summer chinook salmon smolts for release into the Pahsimeroi River and to trap and spawn steelhead adults. Both upper and lower facilities utilize river water from the Pahsimeroi River to culture summer chinook salmon. The river temperatures range from 0°C in the winter to 19°C in the summer. The exposure sites for this hatchery were located in the river water intake canal that provides river water to the earthen ponds (91.5 m x 12.2 m x 1.5 m) at the upper facility and at the effluent screen of the settling pond (91.5 m x 12.2 m x 1.2 m) directly below the earthen rearing ponds (Appendix C).

METHODS

Rainbow trout were obtained from Hayspur and Nampa hatcheries immediately prior to each exposure trial to be used as sentinel fish. These fish averaged 0.96 g to 1.5 g per fish. Each site was supplied with one cylindrical aluminum live-box that measured 47 cm in length x 30.3 cm in diameter. Restricted holding space in the Eagle Fish Health Laboratory wet lab limited the number of experimental units to one per exposure site. The sentinel fish were transported to and from exposure sites in plastic bags with water and oxygen. Each exposure trial consisted of 20 rainbow trout in each experimental unit. These fish were challenged for 10 d, returned to the wet laboratory at the Eagle Fish Health Laboratory, and held in 13°C well water until at least 1,800 Celsius Temperature Units (CTUs) were accumulated (approximately five months). Water temperatures during exposures were monitored with StowAway XTI temperature loggers. River discharge measurements were obtained from the Idaho Department of Water Resources at gaging stations closest to each exposure site. Mean temperature and river discharge were calculated for each exposure.

Following the holding period, fish were euthanized with Tricaine Methane Sulphonate (MS 222), decapitated, and spores enumerated from individual heads using a modification of the pepsin-trypsin digest method (Markiw & Wolf 1974; Burton et al. 2000). Spore counts for each exposure group were ranked and assembled as follows: Negative (below the detection level of 1,667 spores per head); Low (1,667 to 10,000); Moderate (11,700 to 40,000); High (41,700 to 70,000); or Very High (>70,000). These rankings were selected based on previous enumeration results with wild trout. Timing of examination of sentinel fish for spore enumeration by pepsin-trypsin methodology was based on the experience of Eagle Fish Hatchery Lab (Burton et al. 2003). The first survival of MC spores is noticed at 832 CTUs and is characterized by altered or missing spore morphology. A period of increasing spore survival is noticed between 900 and 1,300 CTUs. Spore counts plateau from 1,300–1,600 CTUs. Samples were taken after 1,800 CTUs during these exposure trials to allow maximum spore development and survival. Estimated spore counts per head were achieved by multiplying hemocytometer spore count by 1,667.

Alternate methods for evaluating MC intensity of infection were considered before deciding on spore counts. Triactinomyxon enumeration was considered because this method was rapid, but TAM enumeration does not address actual parasitism, and total counts can be confounded by TAMs from a different species of myxosporean. As such, this methodology was rejected. Histopathology was also rejected as an analysis method for this study because of the amount of time and expense required to process samples. Furthermore, histological techniques view a limited amount of tissue, which could result in a false negative reading. Quantitative Polymerase Chain Reaction was not available upon commencement of these exposure trials. In the future, this technique may become an important tool in future MC research.

SYSTAT 10 (SYSTAT 2000) was used to analyze prevalence data by performing Fisher's Exact Tests (FET) $\alpha = 0.05$ to determine significant differences in monthly MC prevalences between the intake site at PH versus the head box of small raceway four at SFH, and the intake site at PH versus the settling pond site at PH. Spore count data was evaluated for significant differences using Wilcoxon's Rank Sum Tests (WRST) $\alpha = 0.05$ (Ott, 1977). Monthly spore count data were analyzed between the intake site at PH versus the head box of small raceway four at SFH and the intake site at PH and the settling pond site at PH.

RESULTS

Exposure Trials at the Sawtooth Fish Hatchery

Results from the first two exposure trials conducted in February and March of 2000 demonstrated that the infective TAM stages of MC were below detectable levels in the Salmon River water used to service small raceway number four. The average water temperatures during the first and second exposures were 2.6°C and 3.6°C, respectively. The river discharge during both months averaged approximately 110 cfs. Results from the April of 2000 exposure trial demonstrated that MC was detected at a 20% prevalence level, (4 of 20 fish), with 10% Very High and 10% Low. The two Very High category fish averaged above 70,000 spores/head, while the other two fish averaged 7,000 spores/head (Table 1, Figure 1). The mean monthly discharge in April increased to 250 cfs and the mean water temperature of exposure increased to 6.1 C.

Prevalence and intensity of MC infection increased, as did water temperatures and flows, during the month of May 2000 (Table 1, Figure 1). Prevalence of infection was 95% while river discharge reached 1,570 cfs during the exposure period. Water exposure temperatures averaged 7.2°C in May. Thirty percent of the exposed population exhibited a Low intensity of infection with 5,000 spores/head. A Moderate intensity group comprised 45% of the fish exposed and yielded an average spore count per head of 24,000. A High intensity group comprised 15% of the fish used in this trial, with the mean spore count of 50,000/head. One fish was placed into the Very High group of May's exposure (5% of the exposed population) with a spore count of 139,000/head.

Flows increased to an average of 2,190 cfs, average exposure temperatures increased to 11.2°C in June 2000, and prevalence of MC dropped to approximately 87%. Intensity of infection was similar to that observed in May 2000, with 6% of the exposure group in the Low category (7,000 spores/head), 56% in the Moderate category (23,000 mean spores/head), and about 12% in both of the High and Very High categories (54,000 and 109,000 mean spores/head, respectively, for High and Very High categories).

Detected MC prevalence in the month of July 2000 was 80% (Table 1, Figure 1). The average exposure temperature increased to 14.0°C, while the average river discharge decreased to 470 cfs. Intensity of infection decreased with no observations in the High or Very High categories. Seventy percent of the exposed population was categorized in the Low intensity group (5,000 spores/head), while the remaining 10% was placed in the Moderate category (22,000 spores/head).

A rise in both prevalence and intensity of MC infection was realized in August 2000 with 95% of the fish exhibiting MC infections (Table 1, Figure 1). The Very High category comprised

20% of the population with 88,000 spores/head. Spore counts per head were not as high as those observed in May and June exposures, yet all exposure classification categories were represented.

The exposure trials at SFH from September 2000 through January 2001 demonstrated a progressive decline in prevalence and intensity of infection as well as water temperature and river discharge (Table 1, Figure 1). Prevalence declined from 85% in September 2000 to 5% in January 2001. High and Very High categories were not represented in September through January.

Exposure Trials at the Intake Site of Pahsimeroi Hatchery

The results from the February 2000 exposure trial at the intake canal of the upper facility of PH demonstrated MC infection in 67% of the fish (Table 2, Figure 2). Twenty percent of the fish were categorized as having a Very High intensity of infection with an average spore count per head of 102,000. The Low category comprised 30% of the exposed population with a mean of 4,000 spores/head. Fish with a Moderate level of intensity of infection comprised approximately 13% of the study population with an average spore count per head of 15,000. River discharge averaged 329 cfs, and the average temperature was 5.0°C during this trial.

Overall prevalence of infection increased during the March 2000 exposure trial to 85%. Twenty percent of the exposed population was represented in the Low intensity of infection category, with an average of 7,000 spores/head (Table 2, Figure 2). The Moderate category comprised 45% of the fish in this trial period (26,000 mean spores/head). Fish with an intensity of infection classification of High comprised 56% of the exposed fish. The fish categorized as High intensity of infection had an average of 56,000 spores/head during this exposure period. The average river discharge remained about the same at 327 cfs, with an average mean temperature of 6.4°C.

The results provided by the April 2000 exposure trial demonstrated a dramatic increase in the intensity of infection and an increase in prevalence of MC over the March exposure trial (Table 2, Figure 2). The average water temperature increased to 9.2°C, and water discharge decreased to an average of 300 cfs. Prevalence was 100%, with the High and Very High intensity of infection categories represented during this trial. Five percent of the exposed population was categorized as a High level of infection (68,000 mean spores/head), and 95% of the fish were categorized as Very High level of infection (266,000 mean spores/head).

The exposure trials during the months of May 2000 through July 2000 demonstrated a decline in the intensity of infection (Table 2, Figure 2). By July 2000, High and Very High levels of infection were not represented in the fish population even though prevalence was at 100%. The average exposure temperatures climbed to 13.1°C in July 2000, while the mean river discharge dropped to 109 cfs.

A second peak of infection was demonstrated during the August 2000 trial. Prevalence was measured at 100%, while intensity of infection levels were demonstrated in all categories, with the Very High category comprising 65% of the exposed population (194,000 mean spores/head) (Table 2, Figure 2). The average water temperature during exposure peaked for the year at 13.5°C, and average river discharge measured 175 cfs during this time period.

Results from the exposure trial conducted in September 2000 revealed a dramatic decrease in intensity of infection, while prevalence of infection was measured at 80% of the population (Table 2, Figure 2). Only the Low and Moderate levels of infection categories were represented during this exposure trial. The Low category comprised 60% of the fish (4,000 mean spores/head), while the Moderate category consisted of 20% of the fish (19,000 mean spores/head). The average water temperature decreased to 11.9°C during this trial, while average river discharge remained at 175 cfs during the challenge period.

Increases in intensity of infection and prevalence of infection were realized during October 2000. The prevalence of MC was measured at 100%, and all categories of intensity of infection were represented (Table 2, Figure 2). The Very High category comprised 25% of the fish during this exposure (139,000 mean spores/head), while the High level of infection totaled 15% of the population (53,000 mean spores/head). The Moderate category comprised 55% of the fish (28,000 mean spores/head), while 15% of the population was categorized as Low intensity of infection (3,000 mean spores/head). The average river discharge increased during this challenge to 284 cfs, while the average temperature during this exposure trial decreased to 9.3°C.

The last three exposure trials during the months of November 2000 to January 2001 demonstrated a gradual decline in prevalence of MC infection and a decline in intensity of infection for fish at the Pahsimeroi intake site. The December 2000 exposure trial, prevalence 90%, included one fish from the population with 140,000 spores/head, which placed it in the Very High category (Table 2, Figure 2). In January 2001 the average river discharge increased to 321 cfs, and the average temperature during exposure was 3.4°C. Prevalence in January's challenge declined to 80% of the population. Only the Low and Moderate categories were represented in the infected population with 18,000 and 5,000 spores/head, respectively.

Exposure Trials at the Pahsimeroi Settling Pond

Results from the settling pond site at PH from February 2000 to April 2000 demonstrated a gradual increase in MC activity (Table 3, Figure 3). Even though prevalence and intensity of infection dropped in March 2000, the overall tendency was an increase during this period. Pahsimeroi River discharge for the settling pond site was measured at the same location as the intake site throughout the experiment. Consequently, the river discharge values for both exposure sites are the same. Average exposure temperatures gradually increased from 5.0°C in February 2000 to 9.3°C in April 2000. In both February 2000 and April 2000 trials, intensity levels reached the High category (6% and 10.0% of the population in February and April, respectively). The High group in February's challenge had an average 47,000 spores/head, while the High group in April measured 44,000 average spores/head.

The results from the exposure trials from May 2000 to August 2000 demonstrate an increase in prevalence and intensity of infection that peaked in July 2000 (Table 3, Figure 3). July's challenge had a prevalence of 100%, with 55% of the exposed population categorized as Very High (139,000 mean spores/head) (Table 3, Figure 3). The average temperatures during these trials ranged from 10.6°C in May 2000 to 14.1°C in August 2000. The average river discharge ranged from 135 cfs to 175 cfs during the same time period.

The remainder of this experiment, September 2000 through January 2001, demonstrated a decline in the prevalence and intensity of infection. Average temperatures during the exposure trials declined from 12.0°C in September to 3.4°C in January (Table 3,

Figure 3). Prevalence decreased from 80% in September to 25% in January, while the average river discharge increased from 175 cfs to 321 cfs during these months. The September trial had one fish score in the Moderate level of intensity of infection (averaged 42,000 spores/head). After September, intensity levels did not reach the High or Very High categories.

Comparison of Sawtooth Fish Hatchery Site Versus the Pahsimeroi Intake Site

We failed to detect a significant difference in the intensity of infection of MC (WRST, $p \geq 0.05$) at SFH and PH intake sites for exposures conducted in June and September 2000 (Table 4). Significantly higher intensity of infection levels were detected at the PH intake site compared to the SFH site for all other exposures conducted as a part of this experiment. We identified no significant difference in the prevalence of infection of MC (FET, $p \geq 0.05$) at these two sites for the months of April 2000 through September 2000. Significantly higher prevalence levels were identified at PH compared to SFH for all other exposures of the experiment.

Comparison of Pahsimeroi Intake Site Versus Pahsimeroi Settling Pond Site

We failed to detect a significant difference in the intensity of infection of MC (WRST, $p \geq 0.05$) at the two PH sites for exposures conducted in May 2000, September 2000 and November 2000 (Table 4). Significantly higher intensity of infection levels were detected for all other exposures conducted as a part of this experiment. We identified no significant differences in the prevalence of infection of MC (FET, $p \geq 0.05$) in the months of February 2000 through October 2000. Significantly higher prevalence levels were identified for all other exposures of the experiment.

DISCUSSION

Our exposure trials identified monthly fluctuations of MC infectivity and prevalence at SFH and PH river water supplies. The data gathered in these trials demonstrate that the Pahsimeroi River at PH can produce more severe infections of MC than the Salmon River at SFH. An experimental infection in sentinel fish was produced in all 12 months of the experiment at both PH sites, but SFH exposure trials did not produce an experimental infection during the months of February and March 2000. The ability of the Pahsimeroi River to produce a more severe infection is most likely due to the favorable environment for tubificid worms and the heavy influence of springs in this river system.

Both SFH and the PH intake sites had similar monthly fluctuations of MC infectivity. In the spring, as water temperatures increased (April to June for PH and May to June for SFH) to the range of 9.0°C-13.5°C, MC infectivity increased. As temperatures peaked into the range 13.1°C-14.1°C, MC infectivity declined during the month of July. When temperatures dropped to approximately 13.0°C-13.5°C (in August at both sites) there was a second peak of MC infectivity. As temperatures continued to decline in the autumn, both sites had a gradual decrease in MC activity that progressed through January of 2001. River discharge flows do not seem to impact MC activity. At the apex of MC activity SFH is at peak flows (470–2,190 cfs), while PH is at their lowest flows (109–300 cfs).

The PH settling pond site differed from the other exposure sites in that there was only one peak of MC infectivity in June and July 2000 (12.0°C–13.1°C). Prevalence in June was 93% with a mean spore count per head of 125.20 (x1000), while July had a higher prevalence (100%) and a mean spore count per head of 94.18 (x1000). The difference between the PH intake site and the PH settling pond site can be partially explained by operation of PH for chinook salmon production. During the months of February and March, chinook salmon are reared in the earthen ponds between the intake canal and the settling pond sites. These fish could be absorbing the TAM stages of MC and thus filtering MC out of the Pahsimeroi River water source. Chinook salmon were released in April at PH. Subsequently the data demonstrated an increase in MC infectivity at the settling pond site until the next year's production of chinook salmon were ponded in the earthen ponds in September 2000. As temperatures decreased in the Pahsimeroi River in the autumn and winter, a gradual decrease in MC infectivity was demonstrated. The settling pond data also provided some insight on the ability of the settling ponds at MC-positive hatcheries to produce TAMs. Settling pond environments are considered an excellent habitat for tubificid worms. It appears that the settling pond demonstrated increased TAM activity in the Pahsimeroi River water source during June and July 2000, because a dramatic increase in infectivity was observed. At the same time, the PH intake site demonstrated a decrease in infectivity.

RECOMMENDATIONS

From the data produced in these trials, recommendations can be made to improve and renovate PH. A well water source providing 14 cfs of water supply at 10°C would enable the PH staff to culture chinook salmon to the 9.0 cm length that would be refractory to MC infection. Early rearing in concrete raceways would further exclude MC infection activity by eliminating tubificid worm habitat. The PH staff estimates a 9.0 cm average length in chinook salmon cultured in this well water environment would be achieved by May. Even though entry into the Pahsimeroi River water source would not be ideal in May, it would avoid up to six months of exposure to this parasite. If funding is still available, renovation of the earthen ponds used for final rearing could be altered to further exclude MC contact. An artificial lining of the pond or hard surface raceways would further limit TAM-to-fish interaction by placing a barrier between the pond bottom and the water column (fish). Thus, any TAM production by tubificid worms in the earthen ponds would be excluded. Furthermore, a pipe system to bypass the settling pond and release chinook salmon directly into the Pahsimeroi River would improve upon current operation procedures. This release strategy would achieve another avoidance of MC TAMs.

The role of settling ponds at MC positive facilities in maintaining or amplifying MC infections in the natural river is now being questioned. Potential litigation by Colorado Trout Unlimited over settling pond amplification of MC leading to trout population declines downstream of positive facilities has initiated strategies for management of settling ponds. Best Management Practices have been developed for settling ponds of MC positive facilities. Settling ponds should be cleaned and dried at least annually. A capability to alternate settling ponds has been suggested. Daily removal of mortalities and off-site disposal would be essential in maintaining low MC spore introduction into settling ponds along with elimination of stocking settling ponds with susceptible fish. Lining settling ponds would limit tubificid habitat and reduce MC activity.

Due to Idaho Power Company budget constraints, a large renovation of PH may not be feasible, so prioritization is essential in this project. Providing a specific pathogen-free water source at 14 cfs and an enclosed early nursery rearing building that provides enough water and container space to rear 1,000,000 chinook salmon fry to 75 mm are highest priority. These would eliminate exposure to the parasite and attain enough growth to be refractory to whirling disease. The next priority is to renovate the earthen ponds and provide a settling pond bypass pipe for chinook salmon release directly into the Pahsimeroi River. The last phase of the project should address the settling pond improvements. This does not preclude the hatchery staff from addressing such settling pond issues as daily removal of mortalities from the final rearing ponds and elimination of susceptible fish from the settling ponds. Presently, the settling pond is dried and accumulated silt removed on an annual basis.

The SFH already has a specific pathogen-free water source that services early rearing for chinook salmon. An enhancement to that water supply would increase the ability of the SFH staff to avoid MC infection in the fish reared at this facility. There is no pressing need to renovate rearing accommodations for fish at SFH, since all fish at SFH are reared in concrete raceways or artificial tanks. Future modifications at SFH should include altering the one large settling pond into at least two settling ponds. This will allow the SFH staff to alternate the use of the ponds and allow one pond to dry out when not in use. The settling ponds should also be lined. These renovations should reduce TAM production and limit overall MC contribution back to the Salmon River by SFH. Currently the SFH staff removes fish mortalities on a daily basis and does not allow these dead fish to drift into the settling pond. The SFH manager will need to consider drying the hatchery’s settling pond on an annual basis.

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Table 1. Whirling disease data from the exposure trials located at the Sawtooth Fish Hatchery small raceway 4 site, February 2000 to January 2001.

Exposure Dates	Sample N	Average Temperature °C	Average River Discharge (cfs)	Prevalence (Percent)	Mean Estimated Spore/head (X1000)*	Standard Deviation (Spore/Head)	Coefficient of Variation
2/9-19/2000	16	2.6	110	0	0	0	0
3/13-22/2000	20	3.6	110	0	0	0	0
4/2-12/2000	20	6.1	250	20	15.00	48.51	3.23
5/1-11/2000	20	7.2	1,570	95	24.45	31.12	1.27
6/1-102000	16	11.2	2,190	88	33.96	33.69	0.99
7/7-17/2000	20	14.1	470	80	5.42	7.07	1.33
8/1-112000	20	13.0	341	95	36.81	31.87	0.87
9/13-23/2000	20	11.4	304	85	5.15	5.76	1.12
10/20-30/2000	20	7.1	258	55	1.20	1.83	1.53
11/1-11/2000	19	3.3	180	19	7.37	2.42	0.33
12/20-30/2000	15	2.9	123	13	0.25	0.79	3.16
1/22/00-2/1/01	20	3.2	118	5	0.50	2.24	4.48

* Total number of spores/head (including negatives) divided by n.

Table 2. Whirling disease data from the exposure trials located at Pahsimeroi intake canal site, February 2000 to January 2001.

Exposure Dates	Sample N	Average Temperature °C	Average River Discharge cfs	Prevalence (Percent)	Mean Estimated Spore/Head (X1000)*	Standard Deviation	Coefficient of Variation
2/9-19/2000	15	5.0	329	67	23.67	43.57	1.84
3/13-22/2000	20	6.4	327	85	23.67	22.07	0.93
4/2-12/2000	20	9.2	300	100	256.44	179.61	0.70
5/1-11/2000	20	10.3	135	100	58.94	56.44	0.96
6/1-10/2000	19	12.0	133	90	40.62	60.98	1.50
7/7-17/2000	20	13.1	109	100	13.34	5.57	0.42
8/1-11/2000	19	13.5	175	100	141.08	116.22	0.82
9/13-23/2000	20	11.9	175	80	6.25	7.29	1.17
10/20-30/2000	20	9.3	284	100	56.49	60.24	1.07
11/1-11/2000	20	5.8	300	85	9.24	13.30	1.44
12/20-30/2000	20	3.2	311	90	18.58	34.51	1.86
1/22/00-2/1/01	20	3.4	321	80	4.11	4.12	1.00

* Total number of spores/head (including negatives) divided by n.

Table 3. Whirling disease data from the exposure trials located at the Pahsimeroi settling pond site, February 2000 to January 2001.

Exposure Dates	Sample N	Average Temperature °C	Average River Discharge (cfs)	Prevalence (Percent)	Mean Estimated Spore/Head (X1000)*	Standard Deviation	Coefficient of Variation
2/9-19/200	16	5.0	329	75	13.85	13.60	0.98
3/13-22/2000	20	6.4	327	65	5.26	7.69	1.46
4/2-12/2000	19	9.3	300	89	14.67	14.35	0.98
5/1-11/2000	20	10.6	135	100	53.18	26.85	0.50
6/1-10/2000	14	12.3	133	93	125.20	111.67	0.89
7/7-17/2000	20	13.7	109	100	94.18	61.23	0.65
8/1-11/2000	20	14.1	175	100	46.97	67.14	1.43
9/13-23/2000	20	12.0	175	80	9.35	11.15	1.19
10/20-30/2000	13	9.3	284	92	6.72	7.70	1.14
11/1-11/2000	17	5.9	300	76	3.32	5.96	1.79
12/20-30/2000	19	3.3	311	37	1.23	2.52	2.05
122/00-2/1/01	20	3.4	321	25	1.88	6.68	3.55

* Total number of spores/head (including negatives) divided by n.

Table 4. Values for statistical comparisons of whirling disease exposure trials, February 2000 to January 2001. S = Sawtooth headbox of small raceway 4 site; Pa = Pahsimeroi intake site; Pb = Pahsimeroi settling pond site; v = versus.

		Infection Intensity Wilcoxon Ranked Sum Test		Prevalence
Exposure Trial	Month	Z Value	t-test P	Fisher's Exact Test P
SvPa I	Feb. 2000	3.811	P <0.0001	0.000016358
PavPb I	Feb. 2000	2.004	P <0.025	0.704251111
SvPa II	March 2000	5.110	P <0.0001	0.000000026
PavPb II	March 2000	2.565	P <0.010	0.273343056
SvPa III	April 2000	5.207	P <0.0001	0.000000154
PavPb III	April 2000	5.343	P <0.0001	0.230769232
SvPa IV	May 2000	2.150	P <0.025	1.0
PavPb IV	May 2000	0.657	P >0.05	1.0
SvPa V	June 2000	0.249	P >0.05	1.0
PavPb V	June 2000	1.860	P <0.025	1.0
SvPa VI	July 2000	4.184	P <0.0001	0.106029108
PavPb VI	July 2000	5.352	P <0.0001	1.0
SvPa VII	August 2000	3.153	P <0.005	1.0
PavPb VII	August 2000	3.222	P <0.005	1.0
SvPa VIII	Sept. 2000	0.599	P >0.05	0.677318399
PavPb VIII	Sept. 2000	0.655	P >0.05	1.0
SvPa IX	Oct. 2000	5.191	P <0.0001	0.001228501
PavPb IX	Oct. 2000	3.587	P <0.0001	0.459459454
SvPa X	Nov. 2000	3.914	P <0.0001	0.000011553
PavPb X	Nov. 2000	1.119	P >0.05	0.676599681
SvPa XI	Dec. 2000	3.562	P <0.0001	0.000004017
PavPb XI	Dec. 2000	3.512	P <0.0001	0.000652627
SvPa XII	Jan. 2001	4.314	P <0.0001	0.000002210
PavPb XII	Jan. 2001	3.640	P <0.0001	0.001231857

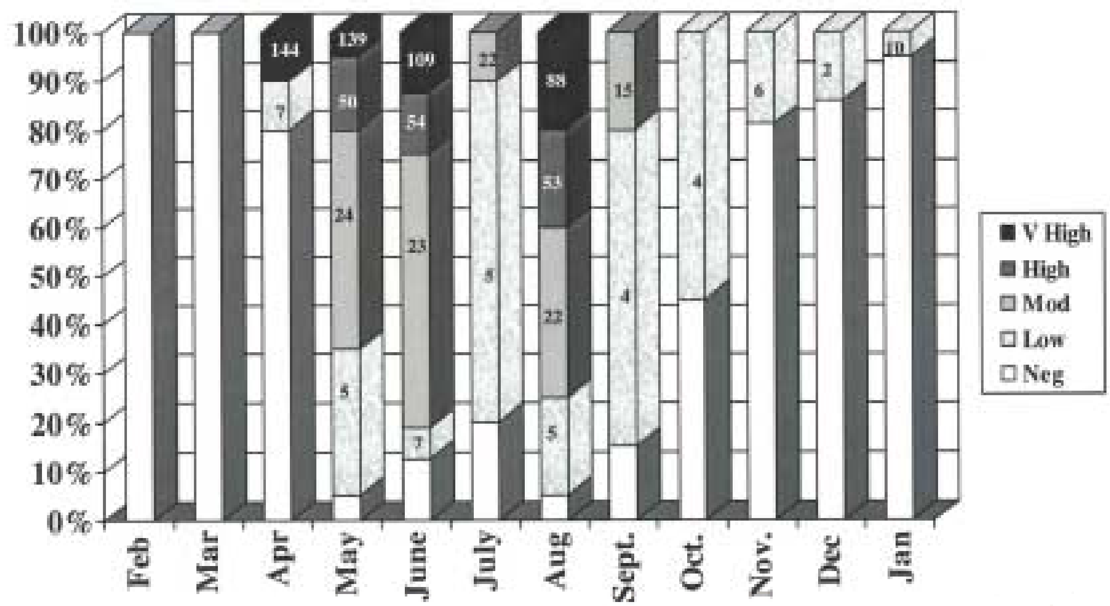

Figure 1. Prevalence and intensity of *M. cerebralis* infection of sentinel rainbow trout exposed for ten days to the Salmon River water supply of Sawtooth Hatchery, February 2000-January 2001. Numbers inside ranked boxes reflect estimated average spore count per category.

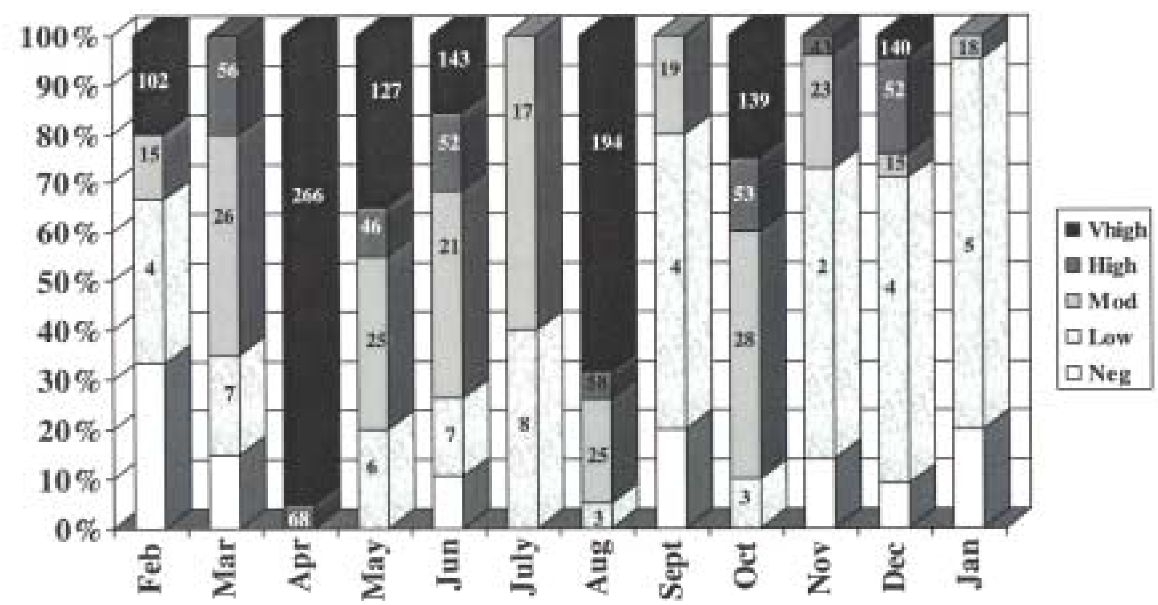

Figure 2. Prevalence and intensity of *M. cerebralis* infection of sentinel rainbow trout exposed for ten days to the river water supply of Pahsimeroi Hatchery, February 2000-January 2001. Numbers inside ranked boxes reflect estimated average spore count per category.

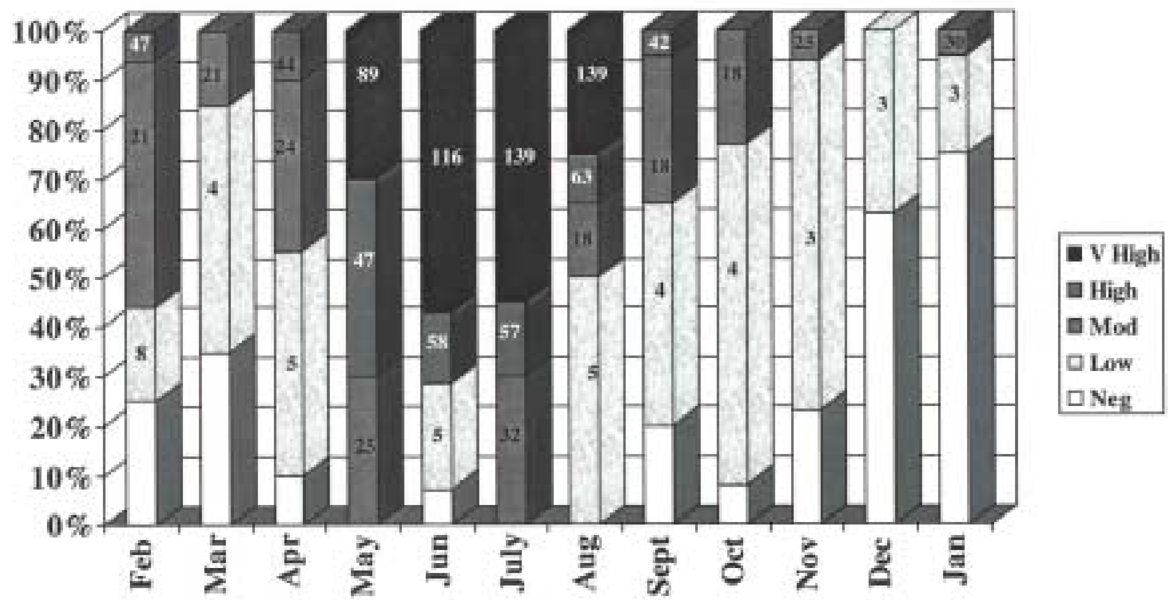

Figure 3. Prevalence and intensity of *M. cerebralis* infection of sentinel rainbow trout exposed for ten days to the settling pond of Pahsimeroi Hatchery, February 2000-January 2001. Numbers inside ranked boxes reflect estimated average spore count per category.

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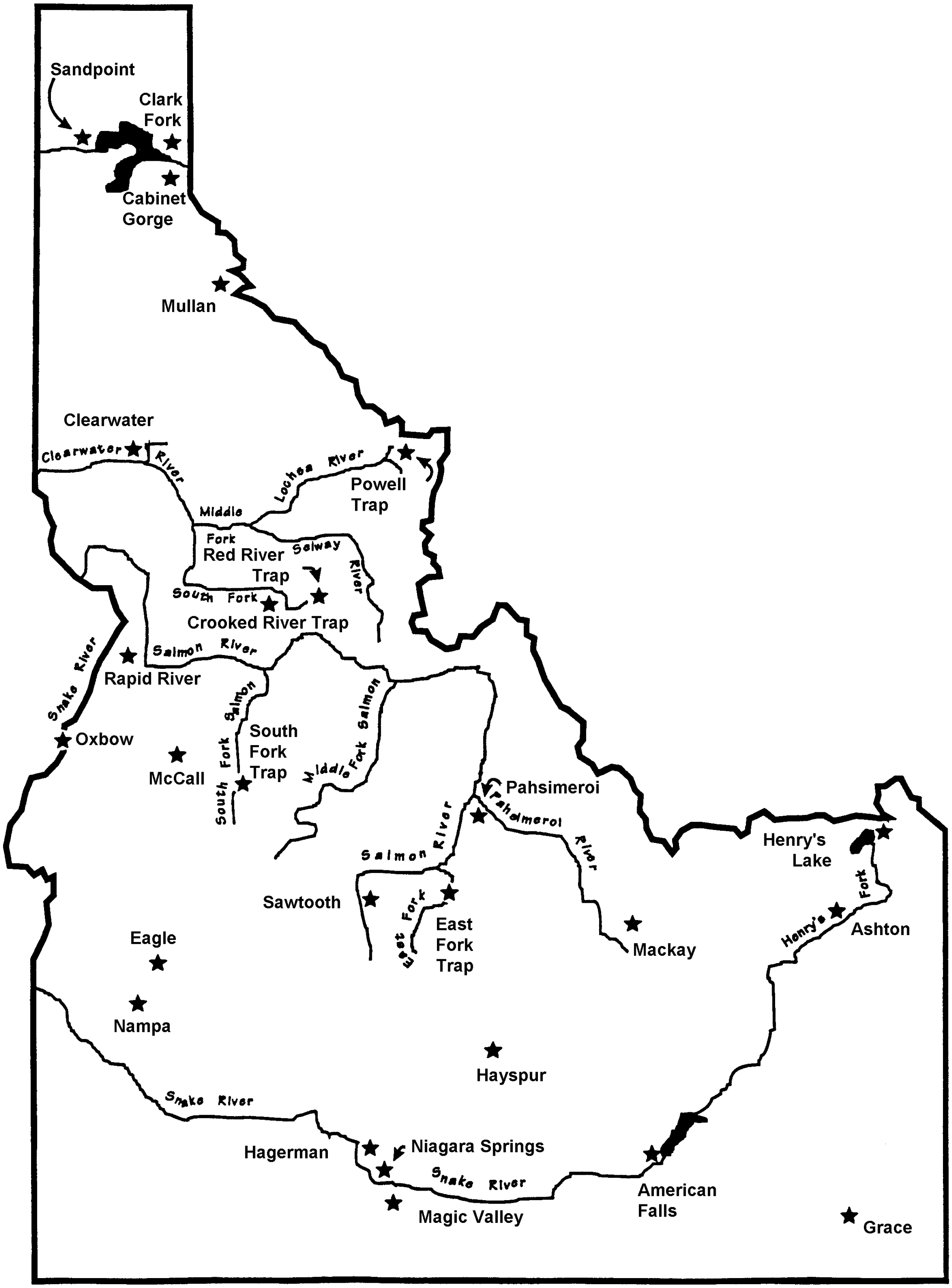
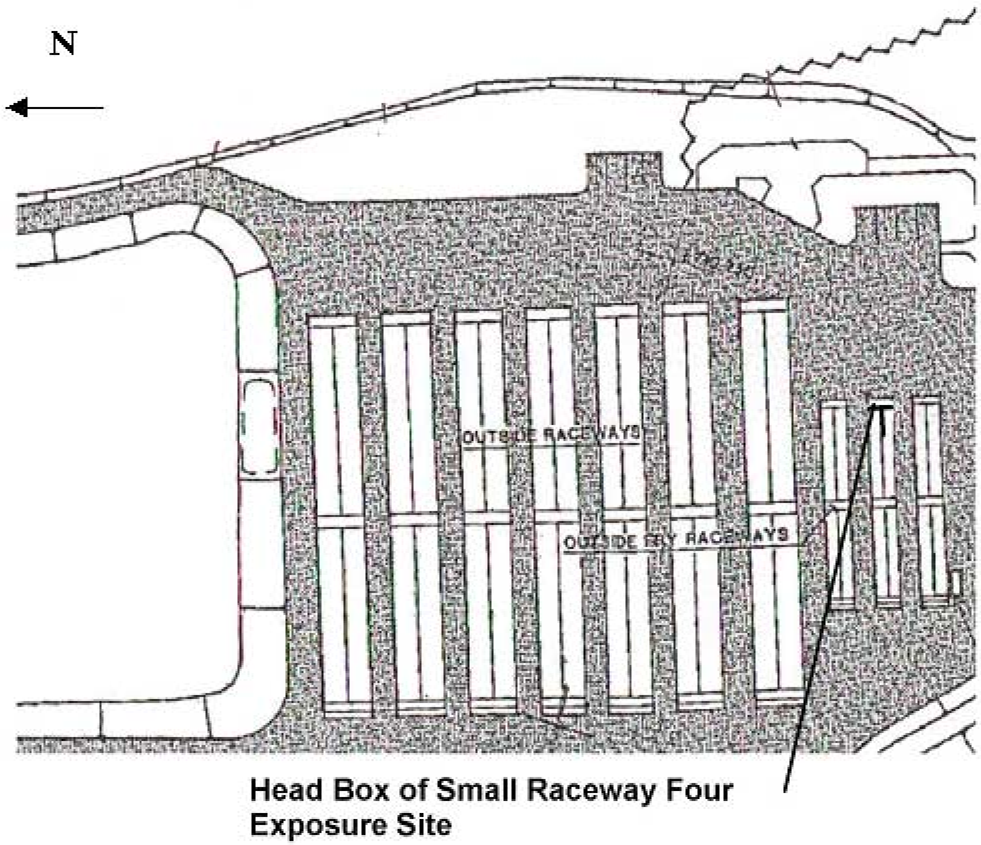
APPENDICES

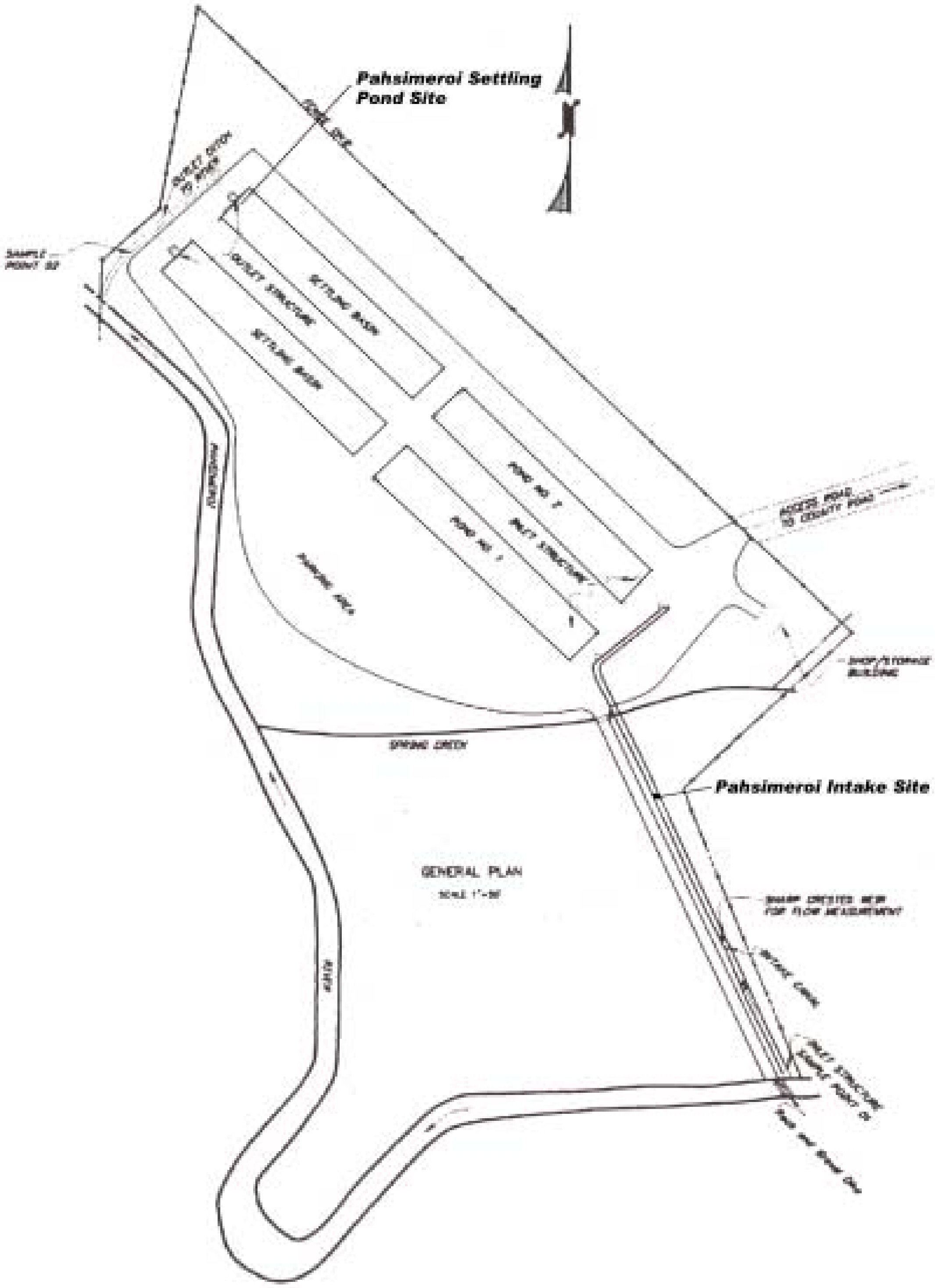
Appendix A. Map of IDFG Fish Hatcheries.



Appendix B. Sawtooth Fish Hatchery Raceways Schematic.



Appendix C. Upper Pahsimeroi Hatchery Schematic.



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